*BioArray*ä *HighYield*ä RNA Transcript Labeling Kit (T7)

Cat. No. 42655-10 10 labeling reactions
Cat. No. 42655-20 20 labeling reactions

For Research Use Only

INTRODUCTION

The ENZO ***BioArray***ä ***HighYield***ä **RNA Transcript Labeling Kit (T7)** has been developed for the production of large amounts of hybridizable biotin-labeled RNA targets by ***in vitro*** transcription from bacteriophage T7 RNA polymerase promoters. Using T7 RNA polymerase and biotin-labeled nucleotides, large amounts of single stranded nonradioactive RNA molecules can be produced ***in vitro***. Because of the nature of transcription reactions, many RNA copies of the template DNA are produced during a short incubation. Because RNA-DNA hybrids have a higher melting temperature than corresponding DNA-DNA hybrids, single-stranded RNA targets offer higher target avidity and greater sensitivity than DNA probes. RNA targets offer selectivity unavailable with DNA targets−being single stranded, they are strand-specific and hybridize more effectively to probes because the target population does not self-hybridize.

RNA transcripts that are labeled with biotin-modified ribonucleotides are used effectively in nucleic acid array assays. The biotin-labeled RNA targets that are hybridized to arrays of DNA probes can be detected by a reporter molecule linked to streptavidin, avidin or anti-biotin antibody. Such a complex can be detected directly, ***e.g.***, by excitation of a fluorophore conjugated to streptavidin, or indirectly, ***e.g.***, using an enzyme conjugate that can produce an insoluble colored precipitate.

The ENZO ***BioArray***ä ***HighYield***ä **RNA Transcript Labeling Kit (T7)** has been formulated and optimized for use with nucleic acid array assays. The kit is available in a 10-reaction size (Cat. No. 42655-10) and a 20-reaction size (Cat. No. 42655-20). Each reaction has been formulated for approximately 1 µg of transcribable cDNA template or a control template such as plasmid DNA (0.4 pmol).

REAGENTS PROVIDED

	Cat. 42655-10 (10 reactions)	Cat. 42655-20 (20 reactions)
Vial 1 10X HY Reaction Buffer	1 x 40 µl	2 x 40 µl
Vial 2 10X Biotin-Labeled Ribonucleotides	1 x 40 µl	2 x 40 µl
Vial 3 10X DTT	1 x 40 µl	2 x 40 µl
Vial 4 10X RNase Inhibitor Mix	1 x 40 µl	2 x 40 µl
Vial 5 20X T7 RNA Polymerase	1 x 20 µl	2 x 20 µl

EQUIPMENT AND REAGENTS REQUIRED BUT NOT PROVIDED

- Water Bath set to 37°C
- DEPC-treated sterile deionized water
- Template DNA Containing T7 promoter

STORAGE

Store all reagents at -20°C, in a freezer that is not self-defrosting.

RNA TRANSCRIPT LABELING PROCEDURES

A. Template Preparation

- cDNA templates should be cleaned up using phenol:chloroform, or an appropriate DNA purification kit, followed by ethanol precipitation. For ethanol precipitation, add ammonium acetate to 2.5M, then add 2.5 volumes of absolute ethanol (-20°C). Immediately centrifuge (≥12,000 x g) at room temperature for 20 minutes. Wash twice with 70% or 80% cold ethanol. After precipitation, spin briefly and aspirate any residual ethanol. Air dry the pellet.
- For control plasmid templates, linearize the plasmid DNA using appropriate restriction enzyme digestion.
- Restriction enzymes that leave a 3' overhang should be avoided because T7 RNA polymerase may transcribe these in a promoter independent manner.
- Template DNA should be purified before adding to the reaction.
- Use only RNase-free water, buffers and pipette tips.

The purity and quality of template DNA is important for high yields of biotin-labeled RNA.

B. RNA Transcript Labeling Reaction

1. Add reaction components to RNase-free microfuge tubes.

Prior to use, spin all components briefly to collect the reagent at the bottom of the tube.

2. Make additions in the order indicated in the following table.

Keep reactions at room temperature while additions are made to avoid precipitation of DTT.

Reagent	Volume
Template DNA	variable to give 1 µg of cDNA or 0.4 pmol (about 1 µg of a 3.8 kb template)
Distilled or deionized water	variable (to give a final reaction volume of 40 µl)
10X HY Reaction Buffer (Vial 1)	4 µl
10X Biotin-Labeled Ribonucleotides (Vial 2)	4 µl
10X DTT (Vial 3)	4 µl
10X RNase Inhibitor Mix (Vial 4)	4 µl
20X T7 RNA Polymerase (Vial 5)	2 µl
Total Volume	**40 µl**

3. Carefully mix the reagents and collect the mixture in the bottom of the tube by brief (5 second) microcentrifugation.

A master mix of all components except template can be made if multiple reactions are to be performed at the same time.

4. Immediately place the tube in a 37°C water bath. Incubate for 4 to 5 hours, gently mixing the contents of the tube every 30-45 minutes during the incubation.

If all materials are free of ribonucleases, the reaction may be continued overnight for a small increase in yield. However, the ribonuclease inhibitor has a relatively short half-life in the reaction.

5. Larger amounts of products can be produced by scaling up all components and volumes. In most scale-up syntheses, the amount of DNA template can be reduced to 0.5-0.8 µg per 40 µl of reaction mixture.
6. Store labeled RNA at -70°C or -20°C if not purifying immediately.

PURIFICATION OF LABELED RNA TRANSCRIPTS

We recommend RNeasy spin columns from QIAGEN for purification of labeled RNA. When eluting the RNA from the column, allow the water eluant to remain layered on the column membrane for two minutes before spinning.

TROUBLESHOOTING

Observation	Suggestions/Comments
Precipitate in the reaction buffer	Centrifuge briefly to remove precipitate before use. After many freeze-thaw cycles, a precipitate may form. The precipitate formation does not interfere with the reaction.
Low yield	The most likely cause of low yield in a transcription reaction is poor quality template. Carry over of phenol will inhibit the reaction. To remove phenol, wash the template twice with 70% or 80% ethanol.
	The presence of excess T7 promoter-containing primers can also decrease yield. Following synthesis of the cDNA template the primers can be removed by precipitating the cDNA with 2.5M ammonium acetate and 2.5 volumes of absolute ethanol. The precipitate should be spun immediately at room temperature for 20 minutes. If other salts are used or if the sample is frozen the primers may also precipitate resulting in their incomplete removal. If interference by excess primers persists, the starting concentration of primers can be reduced. This is recommended when starting with reduced amounts of RNA.
	Some cDNA synthesis reactions may produce cDNA that has been primed with RNA instead of with the T7 promoter-containing oligo primer. This is more likely to occur when starting with total RNA. The RNA-primed cDNA contains no T7 promoter sequence and thus will not be transcribed.

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